Original Research

Molecular Typing of Methicillin Resistant *Staphylococcus aureus* (MRSA) by Ribosomal DNA Spacer PCR (RS-PCR Ribotyping)

M. Soma Sekhar[1]*, T. Srinivasa Rao[1], N. Mohammad Sharif[3] and M. Muralidhar[2]

NTR College of Veterinary Science, Gannavaram, Sri Venkateswara Veterinary University, Andhra Pradesh, INDIA

[1]Department of Veterinary Public Health and Epidemiology
[2]Department of Animal Genetics and Breeding
[3]Department of Veterinary Microbiology, College of Veterinary Science, Tirupati, Andhra Pradesh, INDIA

***Corresponding author:** somasekharmadupuru@gmail.com

Rec. Date:	Oct 01, 2017 10:03
Accept Date:	Jan 21, 2018 13:01
DOI	10.5455/ijlr.20171001100335

Abstract

The present study was delineated to assess the genetic diversity of methicillin-resistant Staphylococcus aureus (MRSA) of canine and human origin. A set of thirteen MRSA isolates obtained from dogs (4), their owners (6) and veterinary students (3) were subjected to Ribosomal DNA spacer PCR (RS-PCR-ribotyping) using primers specific for 16S-23S ribosomal spacer region. Polymorphism was observed with 13 ribotypes discriminated among the 13 MRSA isolates. Phylogenetic grouping was done using PHYLIP software to know the genetic relatedness of MRSA from different sources. Wide genetic diversity and little host specificity was observed among MRSA strains from dogs, dog owners and veterinary students. MRSA isolates from one of the owner and his associated pet dog were present within the same cluster indicating the possibility of zoonotic transmission. No genetic relatedness was observed between MRSA isolates from other dogs and humans. The present findings emphasize the utility of RS-PCR for the detection of polymorphism and to elucidate the genetic relatedness of MRSA strains.

Key words: Dogs, Dog Handlers, MRSA, RS-PCR Ribotyping, Veterinary Students

How to cite: Madupuru, S., Tumati, S., Noorbasha, M., & Metta, M. (2018). Molecular Typing of Methicillin-Resistant Staphylococcus aureus (MRSA) by Ribosomal DNA Spacer PCR (RS-PCR-ribotyping). International Journal of Livestock Research, 8(7), 261-268. doi: 10.5455/ijlr.20171001100335

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a critically important human nosocomial pathogen worldwide that is also an emerging concern in veterinary medicine, being an important cause of a wide variety of hard-to-treat pyogenic infections (Otto, 2012). Methicillin resistance is chromosome

mediated and is related to the mobile genetic element Staphylococcal Cassette Chromosome mec (SCC*mec*) that includes *mecA* gene specifying the production of an abnormal penicillin binding protein 2a (PBP 2a) (Hartman and Tomasz, 1981). Another mechanism of resistance to penicillin in *S. aureus* is production of β-lactamase enzymes (encoded by *blaZ* gene) (Hartman and Tomasz, 1981). Recent studies on MRSA suggest that dogs may act as a significant reservoir for MRSA (Walther *et al.*, 2012). Pet associated persons, pet breeders and veterinarians encompass the primary risk groups that may become colonized from MRSA of canine origin (Loeffler and Lloyd, 2010). Genetic characterization of MRSA isolated from dogs also suggests transmission between humans and dogs, since they carry the same strains that are prevalent in humans (Baptiste *et al.*, 2005 and Weese and van Duijkeren, 2010).

Molecular typing of MRSA is important particularly for the purposes of tracing of outbreaks and subsequent infection control. There are a number of techniques available to type MRSA like Pulsed field Gel Electrophoresis (PFGE), Multi Locus Sequence Typing (MLST), *Staphylococcus* surface protein A (*Spa*) typing, Polymerase Chain Reaction (PCR)-Amplified Ribosomal DNA spacer Polymorphism (RS-PCR-ribotyping) etc. (Moodley *et al.*, 2006 and Walther *et al.*, 2012). Data regarding epidemiology of MRSA of canine origin and its molecular characterization is lacking in India. Hence the present study was carried out with an objective of molecular typing and assessment of genetic diversity of MRSA isolated from nasal swabs of apparently healthy dogs, corresponding dog owners and veterinary students attending canine wards in Andhra Pradesh by RS-PCR ribotyping.

Materials and Methods

Ethical Approval

Ethical approval is not necessary to pursue this study. However, samples were collected without harming dogs. Informed consent was obtained from corresponding dog owners.

Bacterial Reference Strain

The reference strain MRSA (ATCC 25923) was obtained from Hi-Media Laboratories (Mumbai) and maintained at the bacteriology laboratory of Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh.

Source of Bacterial Isolates

A total of 13 MRSA isolates recovered from nasal swab samples of dogs (4/40), corresponding dog owners (6/40) and veterinary students (3/40) attending canine wards of Teaching Veterinary Clinical Complex (TVCC), NTR College of Veterinary Science (Gannavaram) and College of Veterinary Science (Tirupati), Andhra Pradesh, were used in the present study. The identification of each MRSA isolate was carried out by cultural and biochemical tests (Fig. 1) viz. gram positive cocci with yellow colonies on

DOI 10.5455/ijlr.20171001100335

mannitol salt agar, catalase (positive), oxidase (negative), Voges-Proskauer (positive), haemolysis (positive) and coagualse activity (positive), blue colour colonies on MeReSa CHROM agar, cefoxitin and oxacillin resistance (Sneath and Holt, 2001 and Velasco *et al.*, 2005). Microbiological culture media, buffers and all other chemical reagents were procured from M/s. HiMedia Laboratories (Mumbai). All the 13 isolates were confirmed as MRSA by PCR targeting *mecA* and *blaZ* gene (Vannuffel *et al.*, 1995 and Martineau *et al.*, 2000). Oligonucleotide primers used in the present study were custom synthesized from M/s. Bioserve Biotechnologies Pvt. Ltd. (Hyderabad).

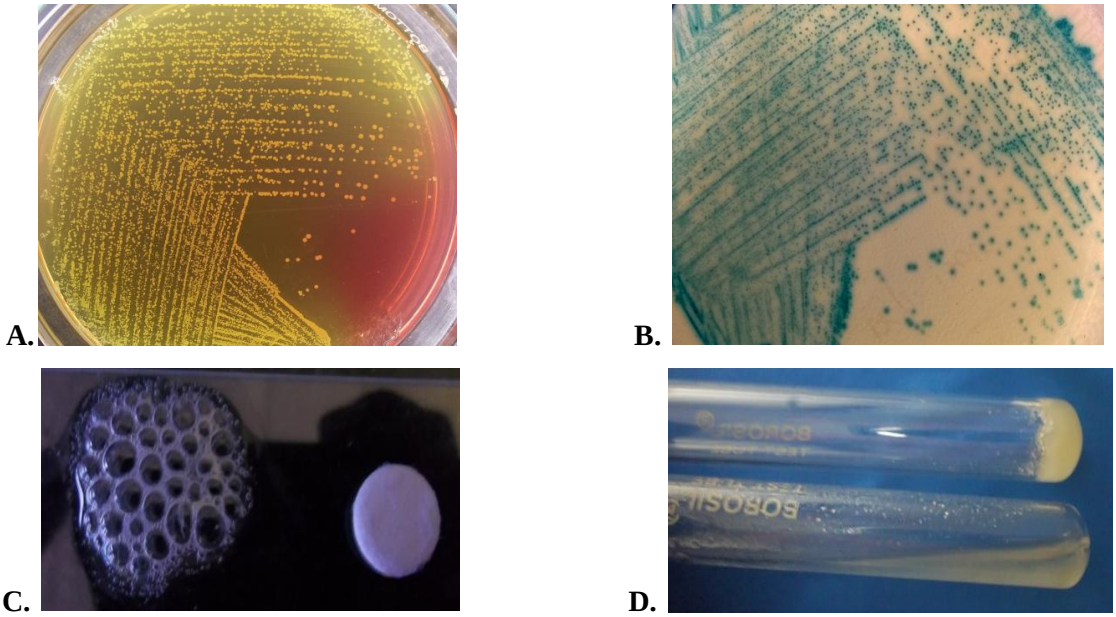

Fig. 1: A). Golden yellow colonies of *Staphylococci* on Mannitol Salt Agar; B). Blue colour colonies of MRSA on MeReSa CHROM agar; C). Catalase (positive) and oxidase (negative) tests; D). Coagulase positive and negative tests.

Ribosomal DNA Spacer PCR (RS-PCR Ribotyping)

Oligonucleotide primers used for amplification of 16S-23S ribosomal DNA spacer region of MRSA from different sources were as described by Jensen *et al.* (1993) and custom synthesized from M/s. Bioserve Biotechnologies Pvt. Ltd. (Hyderabad). A 3.0 µl aliquot of DNA was combined with 2.5µl of PCR reaction buffer with 15mM $MgCl_2$, 1.0 µl of a dNTP mixture (10mM), 1.25µl of each of two 15 base oligonucleotide primers (G1-GAA GTC GTA ACA AGG and L1- CAA GGC ATC CAC CGT) [10 pmol/µl] and 40.0 µl of nuclease free water. This mixture was heated to 94^{o}C for 5 min and 1.0 U of thermostable DNA polymerase was added. PCR assay was performed in Eppendorf (Germany) thermal cycler with heated lid under the following standardized cyclic conditions: 1 min of denaturation at 94^{o}C; 2 min annealing at 55^{o}C; and extension at 72^{o}C for 1 min for 34 cycles and a final cycle of elongation at 72^{o}C for 7 min. The amplified PCR products were subjected to 2% agarose gel electrophoresis under

DOI 10.5455/ijlr.20171001100335

110V for 2 h (Sambrook and Russell, 2001) and bands were visualized under UV trans-illumination using Bio-Rad Gel documentation system.

Scoring of RS-PCR Ribotypes

The PCR amplicons were photographed and analyzed using BIO-RAD Gel Documentation image analysis system (USA) and the supplied image lab software. The position of bands was compared using 100 bp and 1 kb DNA ladder (Genei™, Bengaluru) as an external reference. Binary matrix was generated using binary coding based on presence (1) or absence (0) of a particular band in the given isolate. The binary data was analyzed using dollop programme of PHYLIP (Phylogeny Inference Package) software (version 3.6) with default options and dendrograms were constructed to establish genetic relationship among the MRSA strains from different sources. Clusters were considered at a 70% similarity cut-off and the similarity of band patterns was calculated using the Pearson's correlation coefficient.

Results and Discussion

Over the past few decades, MRSA has emerged as an important pathogen in veterinary medicine. Significant epidemiological and genetic differences exist between MRSA from humans and different animal species (Loeffler and Lloyd, 2010). In order to evaluate the strain diversity and genetic relatedness of MRSA isolates from different sources viz. dogs, dog owners and veterinary students, a total of 13 CoPS isolates were typed by RS-PCR-ribotyping. The resultant DNA amplicons ranged in size from 150 bp to 1000 bp, with 1 to 6 resolved fragments per isolate (Fig. 2).

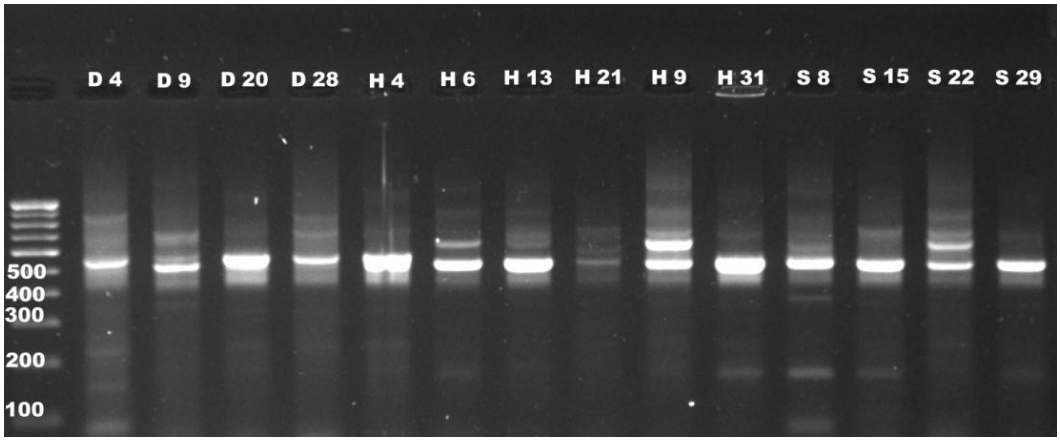

Fig. 2: RS-PCR-DNA finger printing patterns of MRSA isolates from dogs (D4, D9, D20, D28), their owners (H4, H6, H9, H13, H21, H31), veterinary students (S15, S22, S29) and reference strain MRSA, ATCC 25923 (S8).

RS-PCR ribotypes and genetic diversity of MRSA from different sources detected in the present study was given in Table 1. Polymorphism was observed with 13 ribotypes identified among the 13 MRSA

DOI 10.5455/ijlr.20171001100335

strains tested. Molecular typing is essential for differentiation and characterization of MRSA strains from diverse sources (Mulligan and Arbeit, 1991).

Table 1: Genetic diversity of MRSA strains of canine and human origin

Source	No. of MRSA isolates	No. of Ribotypes
Dogs	4	4
Dog owners	6	6
Veterinary Students	3	3
TOTAL	**13**	**13**

Accurate epidemiological typing is of primary importance for detecting routes of transmission of MRSA. Further, the 16S-23S intergenic sequence has been shown to be highly conserved and direct indicator of evolutionary divergence of MRSA (Gurtler and Barrie, 1995). In a study on the comparison of ribosomal spacer DNA amplicon polymorphisms and PFGE for differentiation of MRSA strains, PCR-ribotyping was shown to have almost as discriminatory power as PFGE and suggested for investigation of MRSA outbreaks, being a rapid inexpensive technique that is highly reproducible (Kumari *et al*., 1997). The RS-PCR Ribotyping for characterization of staphylococcal isolates was successfully used earlier by many workers from India and abroad (Oliveira and Ramos, 2002; Dubey *et al.,* 2009 and Reshma *et al.,* 2017). In the present study, phylogenetic analysis discriminated MRSA isolates from different sources of the study into three clusters (Cluster I, Cluster II and Cluster III) for 70% similarity cut-off (Fig. 3).

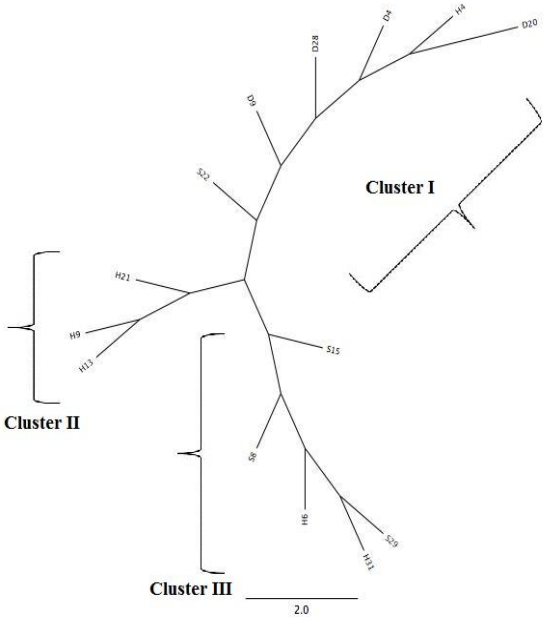

Fig. 3: Cluster analysis of RS-PCR-ribotyping patterns of MRSA isolates from dogs (D4, D9, D20, D28), their owners (H4, H6, H9, H13, H21, H31), veterinary students (S15, S22, S29) and reference strain MRSA, ATCC 25923 (S8) generated using dollop programme of PHYLIP version 3.6.

DOI 10.5455/ijlr.20171001100335

All the four MRSA isolates of dog origin (D4, D9, D20, D28) along with MRSA isolate from a dog owner (H4) and a veterinary student (S22) were grouped under Cluster I; while cluster II comprised exclusively MRSA isolates from dog handlers (H9, H13, H21). Cluster III is intermixed with MRSA isolates from both veterinary students (S15, S29), dog handlers (H6, H31) and reference strain of MRSA (ATCC 25923). Interestingly, MRSA isolates of one of the dog (D4) and its corresponding owner (H4) were present within the same cluster (Cluster I) indicating possibility of transmission of MRSA between dogs and humans (although the direction of transmission could not be proven). These results were in accordance with other study findings that had shown that people and pets can harbor identical strains of MRSA when they share an environment (Morris *et al.,* 2010; Loeffler *et al.,* 2011 and Tarazi *et al.,* 2015). Also, several studies revealed a strong association between human and animal strains of MRSA (Morris *et al.,* 2010 and Tarazi *et al.,* 2015). A limited number of studies have characterized the MRSA isolated from healthy canine samples and have found that the strains found in dogs are characteristics of the strains isolated in human hospitals (O'Mahony *et al.,* 2005; Moodley *et al.,* 2006 and Van Duijkeren *et al.,* 2011). In a study by Zhang *et al.* (2011), MRSA isolates originating from dogs and veterinary staff in Beijing shared similar PFGE patterns, suggesting possibility of cross-transmission of MRSA between pet animals and veterinary staff. This makes the scenario of transfer of MRSA strains from humans to their pets or other animal contacts and subsequent colonisation or infection of the dog. Therefore, while dogs may not be a primary reservoir of MRSA for humans, they do present an important secondary reservoir for re-infection or re-colonisation of humans. Further, intensive daily contact between dogs and their owners was shown to increase the likelihood of interspecies-transmission of CoPS for both sides (Manian, 2003 and Van Duijkeren *et al.,* 2011).

The increasing indiscriminate antibiotic usage has also made canine population a reservoir of MRSA. At the same time, the evolution of new MRSA clones has emphasized the need for infection control practices in animals and humans in close contact. Medical and veterinary staff should appreciate that animals can carry MRSA and cooperate in eliminating infections by implementing guidelines for dealing with MRSA.

Conclusion

The current study demonstrated a wide genetic diversity and little host specificity of MRSA strains among dogs, dog owners and veterinary students. MRSA isolates from one of the owner and his associated pet dog were present within the same cluster indicating the possibility of zoonotic transmission. No genetic relatedness was observed between MRSA isolates from other dogs and humans. In this context, our report is the first from India exploring diversity of MRSA from dogs, owners and professionals. The study emphasized the utility of RS-PCR-ribotyping in the epidemiological investigations for the detection of polymorphism and to elucidate the genetic relatedness of MRSA

 DOI 10.5455/ijlr.20171001100335

strains. Other molecular techniques like Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), *Staphylococcal* protein A (*Spa*) typing and whole genome sequencing are required to establish zoonotic transmission of these strains from animals to animal handlers and vice versa.

Acknowledgements

The Authors thank Sri Venkateswara Veterinary University (SVVU), Andhra Pradesh and Department of Science and Technology (DST), Government of India for the financial support extended in conducting research.

References

1. Baptiste KE, Williams K, Willams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T and Hart CA. 2005. Methicillin-resistant staphylococci in companion animals. Emerging infectious diseases. 11(12):1942.
2. Dubey A, Ghorui SK and Kashyap SK. 2009. Differentiation of *Staphylococcus aureus* strains based on 16S-23S ribosomal RNA intergenic space polymorphism. Indian Journal of Biotechnology. 8: 276-279.
3. Gurtler V and Barrie HD. 1995. Typing of *Staphylococcus aureus* strains by PCR-amplification of variable-length 16S-23S rDNA spacer regions: characterization of spacer sequences. Microbiology. 141(5): 1255-1265.
4. Hartman BA and Tomasz AL. 1981. Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy. 19(5):726-735.
5. Jensen MA, Webster JA and Straus N. 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Applied and Environmental Microbiology, 59(4): 945-952.
6. Kumari DN, Keer V, Hawkey PM, Parnell P, Joseph N, Richardson JF and Cookson B. 1997. Comparison and application of ribosome spacer DNA amplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. Journal of clinical microbiology. 35(4): 881-885.
7. Loeffler A and Lloyd DH. 2010. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiology & Infection. 138(5):595-605.
8. Loeffler A, Pfeiffer DU, Lindsay JA, Magalhaes RS and Lloyd DH. 2011. Prevalence of and risk factors for MRSA carriage in companion animals: a survey of dogs, cats and horses. Epidemiology and Infection. 139(7):1019-1028.
9. Manian FA. 2003. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clinical Infectious Diseases. 36(2):e26-8.
10. Martineau F, Picard FJ, Lansac N, Menard C, Roy PH, Ouellette M and Bergeron MG. 2000. Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis.* Antimicrobial Agents and Chemotherapy. 44(2): 231-238.
11. Moodley A, Stegger M, Bagcigil AF, Baptiste KE, Loeffler A, Lloyd DH, Williams NJ, Leonard N, Abbott Y, Skov R and Guardabassi L. 2006. spa typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. Journal of Antimicrobial Chemotherapy. 58(6):1118-23.

DOI 10.5455/ijlr.20171001100335

12. Morris DO, Boston RC, O'Shea K and Rankin SC. 2010. The prevalence of carriage of meticillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets. Veterinary Dermatology. 21(4): 400-407.
13. Mulligan ME and Arbeit RD. 1991. Epidemiologic and clinical utility of typing systems for differentiating among strains of methicillin-resistant *Staphylococcus aureus*. Infection Control & Hospital Epidemiology. 12(01): 20-28.
14. O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Fanning S and Rossney AS. 2005. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Veterinary microbiology. 109(3):285-96.
15. Oliveira AM and Ramos MC. 2002. PCR-based ribotyping of *Staphylococcus aureus.* Brazilian journal of medical and biological research, 35(2): 175-180.
16. Otto M. 2012. MRSA virulence and spread. Cellular Microbiology. 14(10):1513-1521.
17. Reshma S, Rao TS, Rao TM, Metta M and Sekhar MS. 2017. PCR-ribotyping of bovine and human methicillin-resistant *Staphylococcus aureus.* International Journal of Science, Environment and Technology. 6(3): 1790-1795.
18. Sambrook J and Russell DW. 2001. Molecular Cloning: A Laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press, New York.
19. Sneath PHA and Holt JG. 2001. Bergey's Manual of Systematic Bacteriology, 2nd edition. A Waverly Company, Williams & Wilkins, Springer-Verlag, NY, USA.
20. Tarazi YH, Almajali AM, Ababneh MM, Ahmed HS and Jaran AS. 2015. Molecular study on methicillin-resistant *Staphylococcus aureus* strains isolated from dogs and associated personnel in Jordan. Asian Pacific Journal of Tropical Biomedicine. 5(11): 902-908.
21. Van Duijkeren E, Kamphuis M, Van Der Mije IC, Laarhoven LM, Duim B, Wagenaar JA and Houwers DJ. 2011. Transmission of methicillin-resistant *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics. Veterinary microbiology. 150(3):338-43.
22. Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G and Gala JL. 1995. Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. Journal of Clinical Microbiology. 33(11):2864-7.
23. Velasco D, del Mar Tomas M, Cartelle M, Beceiro A, Perez A, Molina F, Moure R, Villanueva R and Bou G. 2005. Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy. 55(3): 379-82.
24. Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Elnaga YA, Stamm I, Kopp PA, Kohn B, Witte W and Jansen A. 2012. Sharing more than friendship—nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PloS one. 7(4): e35197.
25. Weese JS and van Duijkeren E. 2010. Methicillin-resistant *Staphylococcus aureus* and Staphylococcus pseudintermedius in veterinary medicine. Veterinary Microbiology. 140(3):418-29.
26. Zhang W, Hao Z, Wang Y, Cao X, Logue CM, Wang B, Yang J, Shen J and Wu C. 2011. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and veterinary staff in China. The Veterinary Journal. 190(2):e125-9.

DOI 10.5455/ijlr.20171001100335